ADENOVIRAL REFERENCE MATERIAL WORKING GROUP
PARTICIPATION IN CHARACTERIZATION OF REFERENCE MATERIAL
OTHER CHARACTERIZATION RFP 10.0
Althea Technologies, Inc.

RESIDUAL HOST CELL DNA ASSAY

Althea Technologies, Inc., is a fully equipped BL-2 facility located at 3550 General Atomics Court, Building Number 2, in San Diego, California. Our laboratories and offices are situated in the center of the General Atomics campus, which is within walking distance of the University of California at San Diego, the Scripps Clinic and Research Institute, and the Salk Institute. The General Atomics campus is a highly secure facility comprised of over 35 buildings where a variety of high tech research and development activities are conducted. The campus, with its numerous amenities and access to support services, is an ideal incubator space for biotechnology companies

Althea's gene quantification laboratories occupy 4,500 square feet of space. They were built to our specifications for Good Laboratory Practices compliance and complete prevention of contamination. Different rooms are used for nucleic acid extraction, PCR set up, amplification, and analysis (see attached diagram). The laboratories for nucleic acid extraction and PCR set up are outfitted with seven biosafety hoods equipped with UV lighting, two laminar flow hoods (one for RNA extraction and one for DNA extraction), infectious materials, and high concentration work. Each of our biosafety cabinets is equipped with a dedicated set of five Rainin pipettors. Althea's laboratories have multiple high-speed, refrigerated microfuges manufactured by Beckman and Eppendorf. To perform OD readings for nucleic acid quantitation, Althea use a Molecular Devices plate spectrophotometer. The gene quantification laboratory is equipped with a Packard multiprobe II liquid-handling robot for automating DNA extraction and PCR set up. The cDNA's are prepared using one of our two Perkin Elmer Biosystems 9600 thermocyclers. Tissue and RNA samples are archived in two -80^{o}C Forma freezers. DNA samples are stored in -20^{o}C freezers. The tissues that Althea receives are homogenized with either an Omni powergen or a Savant fast prep system FP120 tissue homogenizer. The quantitative PCR data are generated on one of three PE Biosystems™ 7700 sequence detection systems using TaqMan™ chemistry

Althea Technologies provides quantitative PCR based host cell assays for plasmid, viral preparations and protein preparations. Our sensitive assays have detected down to 1 pg of contaminating host cell DNA from various DNA species including E.*coli*, human, mouse, primate and hamster. The limit of quantitation is 1 pg of host cell DNA with a range of quantitation from 100 ng through 1pg of host cell DNA. Degradation of the contaminating fragment is confirmed using three overlapping PCR primer sets. In parallel, samples are confirmed to be free of PCR inhibitors by spiking with known sequence. As always, gene quantification assays performed at Althea are GLP compliant.

Althea Technologies has four ABI Prism 7700 Sequence Detection systems that are calibrated every four months with the next calibration due 7-27-01**.** Each machine is under a full service contract with ABI to that includes preventative maintenance and immediate

access to service engineers. All other equipment used in GLP studies is calibrated either annually or semi-annually with calibration due dates scattered throughout the year.

Cross contamination issues are addressed by spatial separation for each step of the assay from extraction to PCR the mandatory use of aerosol resistant tips and equipment designated to a specific workstations. PCR workstations and equipment are decontaminated with bleach solution, rinsed with alcohol and then treated with UV light.

Althea has performed over 120 residual host cell contamination studies examining several hundred samples. Over 90% of these studies were adenoviral. Due to confidentiality of our service to our customers, specific data cannot be included in this proposal.

Material required:

1) Host cell line – a minimum of $1x10^7$ cells are required to produce a standard curve against which the test article is quantified.

2) Adenovirus preparation (test article) estimated to contain a minimum of 5 ì g of DNA.

Upon receipt of the host cell line and the test article, Althea Technologies is prepared to begin testing immediately. The sponsor will have preliminary results within ten business days and a full report issued within 15 business days. Data will be reported as pg or ng of host cell DNA per ì g of viral DNA. Althea Technologies is prepared to begin testing in September and the market value to performing this testing is $6,200.00.

Personnel

François Ferré, Ph.D.
Chief Executive Officer and Chief Scientific Officer

Dr. François Ferré is a founder, CEO and CSO of Althea Technologies. He has over fifteen years of experience in cancer research and HIV clinical development and has held positions at Cytometrics and the Immune Response Corporation. Dr. Ferré is a leader in gene quantification with over ten years of experience in the field. He has published several authoritative reviews on the topic of gene quantification, co-edited a book on PCR with Dr. Kary Mullis and Dr. Richard Gibbs and edited a book focused entirely on the subject of Gene Quantification. Dr. Ferré received his Ph.D. in Molecular Oncology from the Pasteur Institute in Lille, France and did his post-doctoral training at the University of California, San Diego.

Study Director – Christopher Duffy

Mr Duffy has over fifteen years of experience managing the operation of clinical and manufacturing laboratories including operation of a biosafety level III laboratory conducting phase III clinical research for an HIV vaccine trial. From 1986-1988 he functioned as Production Lead at the manufacturing lab facility of Cytotech, Inc. From 1988 to 8/2000 he was a Senior Manager at The Immune Response Corporation where he managed a cGMP manufacturing laboratory developing and validating manufacturing and QC methods for vaccine products. He was also responsible for clinical research activities associated with the company's HIV vaccine clinical trials including protocol development and data evaluation for pre-clinical and Phase I-III clinical trials. Mr. Duffy holds a Bachelor of Arts degree in Biology from the University of California, San Diego.

Laboratory Manager – Holly Trotter

Mrs. Trotter has over 17 years of laboratory experience in research, diagnostic testing and assay development; 12 of which were spent in Agriculture Canada's biosafety level III facilities. From 1988-1995 she was a research associate for the virology department of Agriculture Canada's Animal Disease Research Institute where she developed several ELISAs that are now utilized both nationally and internationally as diagnostic tools. From 1995-1997, Mrs. Trotter functioned as Senior Technologist in Agriculture Canada's Foreign Animal Disease diagnostic testing lab where she was responsible for sample testing, data analysis, and supervision and training of technologists and foreign scientists. Early in 1997, Mrs. Trotter moved to Winnipeg, Canada to be the technical liaison in the final building stages of a new biosafety level III and IV facility for Agriculture Canada. Once the laboratory was certified, Mrs. Trotter was the manager in the BSL III laboratory coordinating assay transfer under QA conditions, hiring and training of personnel and sample testing and data analysis. Upon moving to San Diego in 1999, Mrs. Trotter held the position of laboratory coordinator in the virology/immunology lab at San Diego Zoo's Center for the Reproduction of Endangered Species until joining Althea.

Protocol:

QUANTIFICATION OF RESIDUAL HOST CELL DNA IN VIRAL PREPARATIONS

1.0 PURPOSE

The purpose of this protocol is to describe the assays for quantification of residual cellular DNA in viral vector preparations using quantitative PCR.

2.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

2.1 Test Articles:

2.2 Controls

Positive: DNA standards, and test article spiked with 10 pg of an exogenous plasmid to test for PCR inhibition.

Negative: No Template Control (NTC) - PCR reagent control.

2.3 Determination of Strength, Purity, and Concentration.

The Sponsor is responsible for determination and documentation of the analytical purity, composition, and stability of the test article. Retention of reserve sample from each batch of test article is the responsibility of the Sponsor.

3.0 TESTING FACILITY AND KEY PERSONNEL

Name: Althea Technologies, Inc.
Address: 3550 General Atomics Court, Bldg. 2
San Diego, CA. 92121

Study Director: Chris Duffy

4.0 TEST SYSTEM

The detection of DNA sequences by PCR is a standard procedure where a specific fragment of DNA is amplified in vitro to generate many more copies of the original DNA fragment. In the Real-Time modification of the technique, a sequence-specific fluorescent probe present in the PCR reaction detects the amplification product. In the presence of amplification sequences, the probe releases a fluorescent signal.

5.0 EXPERIMENTAL DESIGN AND METHODOLOGY

5.1 DNA will be extracted from the test articles as described in Althea SOP 3-GN-015, *Quantitation of Host Cell DNA Contaminant in Adenoviral DNA Preparations*. Up to one half (0.5) μg of DNA will be analyzed in each PCR.

5.2 PCR amplification will be performed on DNA using oligonucleotide primers and fluorescent probes described in Althea SOP 3-GN-015, *Quantitation of Host Cell DNA Contaminant in Adenoviral DNA Preparations*. To estimate the size of the remaining host cell DNA, three different PCR reactions amplifying 18S rDNA are performed. These assays produce overlapping amplicons of increasing size. In the presence of target sequences, the primers will produce a specific amplification product that is detected by the target-specific fluorescent probe present in the reaction. Each 18S PCR run will include an NTC, serial dilutions of DNA (standards), the test articles and the test articles spiked with a known amount of host cell DNA.

5.3 To determine presence/absence of PCR inhibitors in the sample, test articles and a water control are spiked with 10 pg of exogenous plasmid and amplified using the exogenous plasmid control assay protocol.

5.4 PCR amplification and fluorescence detection will be performed using an ABI PRISM 7700 Sequence Detection System.

6.0 CRITERIA FOR DETERMINATION OF A VALID TEST

6.1 Acceptability of a run is determined by the following criteria:

6.1.1 The correlation coefficient of the standard curve (r^2) must be 0.980 or greater.

6.1.2 The standard curve must include the 1 pg, 10 pg, 100 pg, 1ng, and 10ng standards.

6.2 Acceptability of the test sample under analysis is determined by the following criteria:

6.2.1 The difference in the C_T values of the duplicates that are in the quantitative range must be less than 1 C_T.

6.2.2 The test article spiked with exogenous plasmid must have a C_T value less than exogenous plasmid alone + three C_T, indicating no PCR inhibition.

7.0 EVALUATION OF ASSAY RESULTS

7.1 If both of the C_T values of the sample are 45, or if the Sample Mean C_T plus 1 StDev is greater than the Lower Limit of Detection Mean C_T (LOD C_T), it will be reported as Lower than Limit of Detection (LLD).

8.0 RETESTING POLICY

Runs or samples that do not meet the acceptability criteria will be repeated if sufficient sample is available.

9.0 REPORT

The final study report will be provided to the Sponsor and will include a summary evaluation of the results.

10.0 RECORDS AND ARCHIVES

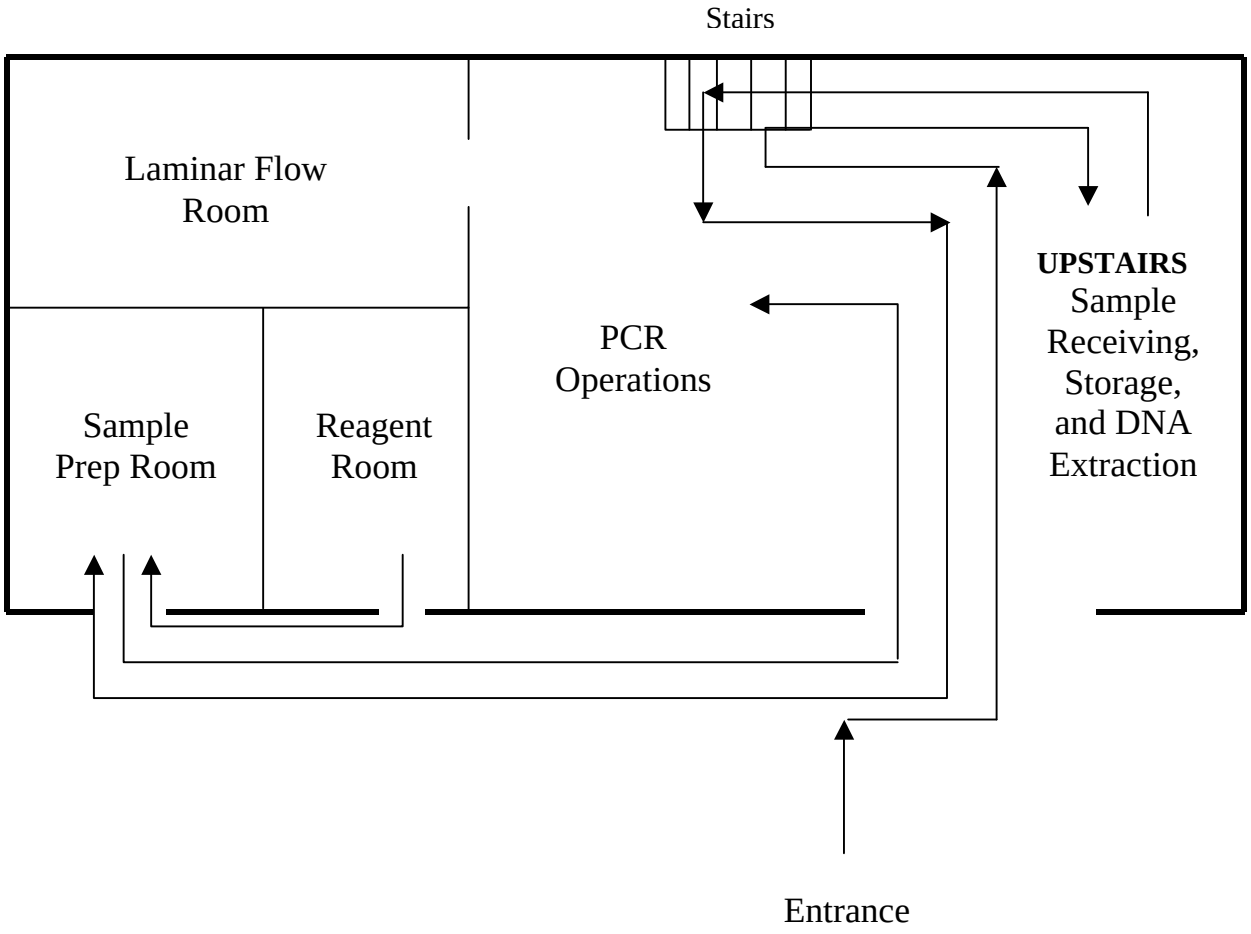
All raw data, the protocol, and a copy of the final report will be maintained according to Standard Operating Procedure 3-QA-011, *Data Recording and Storage*, and kept by the Althea Technologies Quality Assurance Department.

11.0 (GLP STUDY) REGULATORY REQUIREMENTS/ GOOD LABORATORY PRACTICE

This study will be conducted in compliance with U.S. Code of Federal Regulations Good Laboratory Practices (21 CFR, Part 58).

12.0 APPROVAL

PCR Laboratory – Sample Flow



Bid Submission Form
Participation in Characterization of Reference Material – Other Characterization
RFP 10.0

Please complete the following fields:

Contact Information – RFP 10.0

*Contact Individual:	Valerie Mc Donnell/Christopher Duffy
Institution:	Althea Technologies, Inc
Address:	3550 General Atomics Court, Bldg 2 San Diego, CA 92121
Phone Number:	858-455-2183
Fax Number:	858-455-2188
Email Address:	vmcdonnell@altheatech.com

***If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

Please indicate if your institution is also submitting proposals for the other activities:

- ☐ Determination of Particle Concentration
- ☐ Determination of Infectious Titer
- ☐ Short-term/Field Stability Studies
- ☐ Long-term Stability Study
- ☐ Donation of Supplies/Other Services for Characterization Phase